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VARIABILITY IN FLASH CHARACTERISTICS OF A BIOLUMINESCENT COPEPOD

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Abstract

Bioluminescence of the copepod, *Pleuromamma xiphias,* was investigated with an optical multichannel analyzer (OMA) to measure emission spectra, an integrating sphere–photon counting detector system to determine flash kinetics and quantum emission, and an ISIT video system to image spatial patterns of emission.

Light emission was in the blue spectral region, with maximum emission at approximately 492 nm. Spectral waveforms were unimodal, or bimodal with the secondary peak at 472 nm.

Flashes in response to a single stimulus consisted of two components: a fast component attaining maximum intensity in under 100 ms, and a slow element which peaked after 600 ms. The fast component originated from thoracic and abdominal light organs while the slow component represented a large expulsion of luminescent material from the abdominal organ only. Both components exhibited first order exponential decay although the decay rate of the fast component was approximately one order of magnitude greater. The typical flash response to a single stimulus exhibited a response latency of 30 ms, initial rise time of 87 ms, duration of 2.4 s, and quantum emission of 1.4×10^{10} photons flash^{-1}. Quantum emission increased with increasing stimulus strength.

Both response waveform and total quantum emission were affected by the frequency of electrical stimuli. Stimulation at 1 Hz generated the greatest luminescence, averaging 1.1×10^{11} photons response^{-1} for 11 s emissions. Higher rates of stimulation decreased total quantum emission and response episode duration, and resulted in greater temporal summation of the emission waveform.

Variability in flash characteristics due to electrical stimulation suggests a versatility of luminescent displays *in situ.*

Introduction

Recent bathyphotometer measurements in the Sargasso Sea suggest that most stimulable bioluminescence in epipelagic waters originates from zooplankton, principally crustaceans such as euphausiids, ostracods, and copepods, as well as other organisms such as larvaceans and radiolaria (Swift *et al.,* 1983, 1985). Copepods represent approximately 70% of the zooplankton specimens in the upper 200 m of the Sargasso Sea. Calanoid copepods are more abundant than other copepods (Deevey, 1971) and include many luminescent species (Herring, 1978, 1985).

The secreted bioluminescence of copepods originates from multiple glands (Giesbrecht, 1895; Clarke *et al.,* 1962), and presumably functions as part of an escape

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Abbreviations: OMA—optical multichannel analyzer, MCA—multichannel analyzer, S/N—signal to noise ratio, FWHM—full width at half maximum amplitude, PMT—photomultiplier tube.

response from predators (David and Conover, 1961; Buck, 1978; Young, 1983). Flashes can be induced by mechanical, electrical, photic, or vacuum (presumably acting as mechanical) stimulation, as well as by the presence of potential predators (David and Conover, 1961; Clarke *et al.,* 1962; Barnes and Case, 1972; Lapota and Losee 1984; Herring, 1985; Yevstigneyev, 1985). After a brief latency following the stimulus, copepods respond with a flash that rapidly rises to maximum intensity and lasts from less than 1 s to more than 10 s.

A striking aspect of copepod bioluminescence is the variability in flash waveforms and kinetics (David and Conover, 1961; Clarke *et al.,* 1962; Barnes and Case, 1972). This makes it difficult to identify trends in flash responses due to experimental manipulations or other factors. Only in coastal ostracods (Morin and Bermingham, 1980; Morin, 1986) has the variability in flash responses been correlated with different luminescent behaviors.

Our study examines variability in the spectral and temporal characteristics of bioluminescence of the calanoid copepod, *Pleuromamma xiphias.* Members of the genus *Pleuromamma* are numerous throughout the year in the Sargasso Sea (Deevey, 1971) and at times it is one of the dominant genera (Fish, 1954). They are active vertical migrators (Roehr and Moore, 1965), ascending from daytime depths of 350 m to epipelagic depths at night where they contribute to measured bioluminescence (Swift *et al.,* 1983, 1985). The flash of *P. xiphias* is similar to that of other members of the Metridiidae and is readily elicited by electrical pulses (Clarke *et al.,* 1962; Yevstigneyev, 1985). Our results indicate that the nature of the electrical stimulus dictates the type of flash response observed, and suggest that complex neural or other factors play significant roles in regulating the kinetics and quantum emission of the flash response.

Materials and Methods

Adult specimens of *Pleuromamma xiphias* (Giesbrecht, 1889) were collected and studied during the April, 1985, Biowatt cruise aboard the RV Knorr, and during a subsequent cruise on the RV Endeavor in May, 1987, from stations between 28° and 35° N 70° W in the Sargasso Sea. Plankton nets with 333 μm mesh and 0.5 or 1 m mouth diameters were towed at night for 20 min at depths of 80–100 m. Seawater temperatures at these depths ranged from 18–22°C (T. Dickey, pers. comm.). Specimens were sorted and maintained in filtered seawater in darkness until use. All experiments were performed at room temperature (22 ± 2°C) within 10 h of collection. Subsequently, specimens were individually preserved in 4% formalin for later identification.

Spectral measurements

Bioluminescence emission spectra were measured with a Princeton Applied Research optical multichannel analyzer (OMA) system. The OMA detector (EG&G PARC Model 1420) consists of a linear array of 700 intensified photodiodes which simultaneously collects the light signal across a 350 nm spectral window of a polychromator. The OMA system has the requisite high sensitivity, high resolution, and fast response time necessary for registering dim, brief bioluminescent emissions. Details of OMA operation and calibrations have been previously described (Widder *et al.,* 1983).

Specimens were suspended in a drop of filtered seawater between a pair of tungsten electrodes and stimulated at 20 Hz for approximately 4 s with 10 V, 5 ms duration monophasic pulses from a Grass model S44 stimulator. Bioluminescence was

focused onto a 1 mm entrance slit to the polychromator by quartz optics and was integrated over a period of 1–2 s by the OMA system. Spectra with signal to noise ratios (S/N) less than 30 were not used (Widder *et al.*, 1983).

Measurements of flash kinetics and quantal output

The temporal characteristics of bioluminescence were measured with an integrating sphere–photon counting detection apparatus. An integrating sphere is considered critical to precise measurements of quantum emission from sources that may not emit isotropically, *e.g.*, most organisms with photophores. Single specimens were suspended in a drop of filtered seawater between a pair of tungsten electrodes while enclosed in a 10 inch diameter integrating sphere (Labsphere, Inc.). The inside surface of the sphere is coated with white Polane polyethylene paint to ensure maximum reflectance (97% reflectance at 500 nm) and minimize damage to the reflector surface from contact with seawater. A baffle between the source and detector assured that only light that had undergone multiple reflections within the sphere was measured. Bioluminescence was detected by a photon counting RCA No. 8850 photomultiplier tube, operating at −1700 V with a calibrated discriminator setting of −0.315 V, that viewed the interior of the sphere through a 4 cm diameter port. The entire apparatus was calibrated for photon emission both before and after the cruise with an Optronic Laboratory model 310 calibration source referenced to an NBS standard. Quantum calibration took into account not only the spectral responsivity of the integrating sphere and photomultiplier tube but also the spectral emission of bioluminescence of *P. xiphias* as measured by the OMA. The calibration of the system was frequently checked at sea with a C^{14} phosphor (I-Lite, 0.05 mCi) referenced to the Optronics source.

Bioluminescence was stimulated by single or repetitive electrical pulses at various frequencies, while the photomultiplier signal was monitored for either 8 or 20 s with an Ortec No. 776 counter/timer and a Norland No. 5400 multichannel analyzer (MCA). Flash waveforms displayed on the MCA were either directly photographed or videotaped. Printed copies of the flash waveforms were later obtained from the video record after processing by a Megavision model 1024XM image analysis system. Flash kinetics were derived from the printed waveform on a Summagraphics digitizing pad.

Flash characteristics are defined as follows: response latency = time from presentation of the stimulus to beginning of the response; total rise time = time from beginning of the response to maximum intensity of emission; 50% decay time = time from maximum intensity to an intensity one-half that value; total flash or response duration = time from beginning to end of response; response episode = light emission during repetitive stimulation; (total) quantum emission = total integrated photons of response episode; maximum response = response with greatest total integrated photons.

Image intensification

Individual specimens were placed between two metal electrodes in a clear leucite chamber containing approximately 2 ml of filtered seawater. The chamber was enclosed in a sealed box with white reflective internal surfaces. Bioluminescence was viewed from above through a port in the box with an intensified SIT video camera (Dage-MTI Model 66) fitted with a 105 mm Nikon F/4.0 lens. A photon counting detection system (described above) obtained simultaneous measurements of flash waveforms and kinetics. The photon counting tube viewed the interior of the box

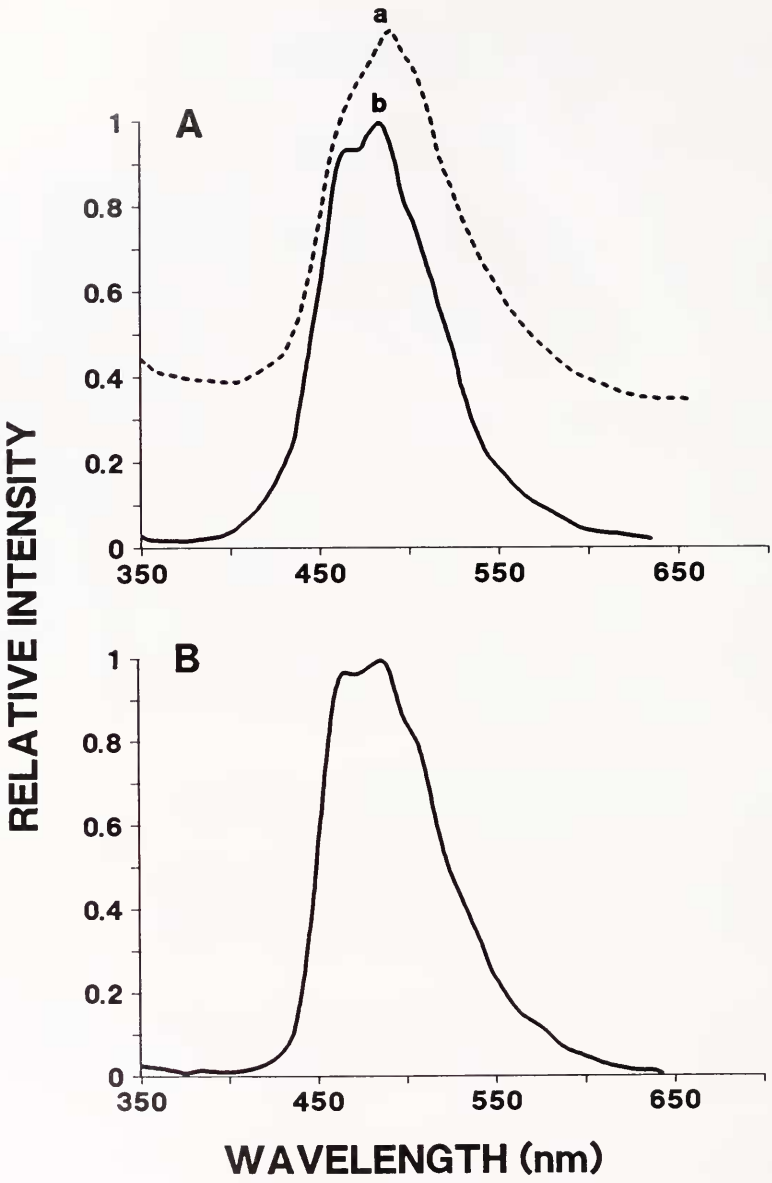


FIGURE 1. Mean emission spectra of the bioluminescence of *Pleuromamma*. (A) *Pleuromamma xiphias* spectra are displayed on the same intensity scale but are vertically displaced for clarity. (a) Unimodal spectral distribution (dashed line) representing emissions from three specimens; max = 493 nm, FWHM = 83 nm, S/N = 103. (b) Bimodal spectral distribution (solid line) from four specimens; max = 492, 472 nm, FWHM = 74 nm, S/N = 97. (B) Spectral distribution representing two specimens of *P. abdominalis;* max = 486, 465 nm, FWHM = 75 nm, S/N = 70.

through a 4 cm diameter port, and measured only reflected bioluminescence. Quantum calibration of this system was not performed.

Light production was stimulated electrically by single or repetitive pulses. The

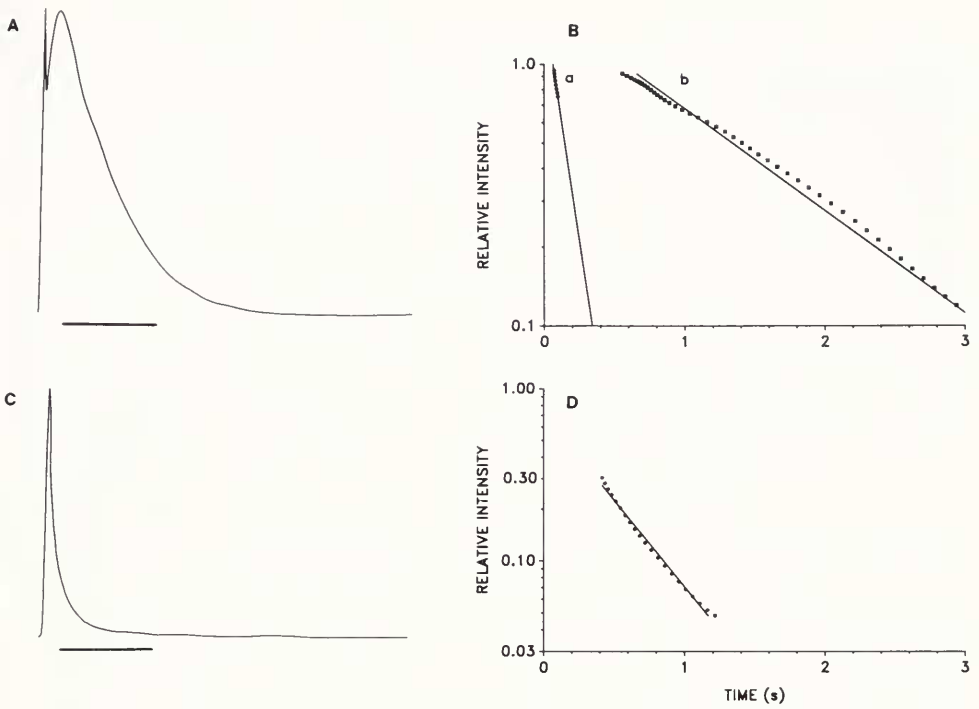


FIGURE 2. Luminescent responses of two specimens of *Pleuromamma xiphias* to a single 10 V, 5 ms duration electrical stimulus. (A) Typical flash response exhibiting fast and slow components. The relative intensity of emission is displayed with time; time bar = 1 s. (B) Decay kinetics of both components of the flash displayed in (A). Relative intensity (log scale) is shown as a function of time. For each component the slope of the calculated linear regression (solid lines) reflects the rate of exponential decay (refer to text). Decay rate of the fast component (a) was −8.3 while the decay rate of the second component (b) was −0.9 (R = 0.99 for each). (C) Flash lacking the second component. As in (A) except that the vertical scale is magnified 10 times. (D) Decay of light emission of the flash displayed in (C). As in (B). The decay rate was −2.3 (R = 0.99).

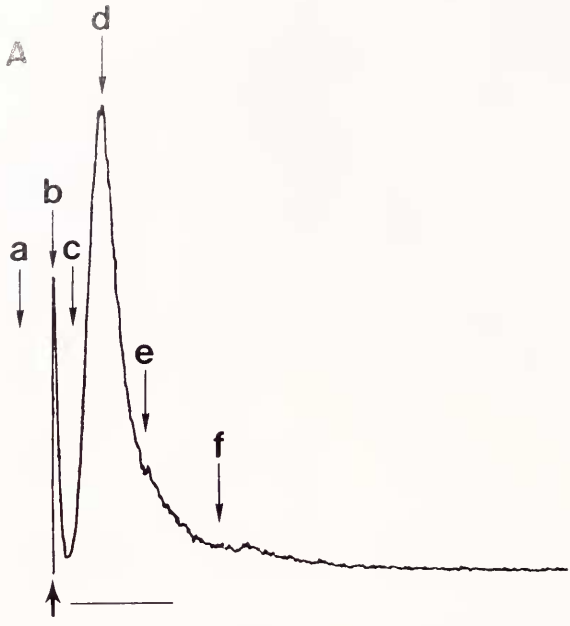
methods of collection and analysis of data were identical to those described in the previous section.

RESULTS

Spectral characteristics

Bioluminescence emission spectra from 7 specimens of *Pleuromamma xiphias* were centered in the blue region of the visible spectrum with maxima at approximately 492 nm. Two types of spectral distributions were measured; about half of the specimens produced unimodal spectra while the others generated bimodal spectra (Fig. 1). Regardless of the spectral shape, the dominant emission was at 492–493 nm while the short-wavelength 472 component was present either as a subpeak or shoulder.

Bimodal emission spectra were also measured from the two specimens of *P. abdominalis* tested. They differed only slightly from those of *P. xiphias,* having maximal emission at 486 nm and a short-wavelength subpeak at 465 nm (Fig. 1). Neither species gave evidence of sexual differences in spectral emissions.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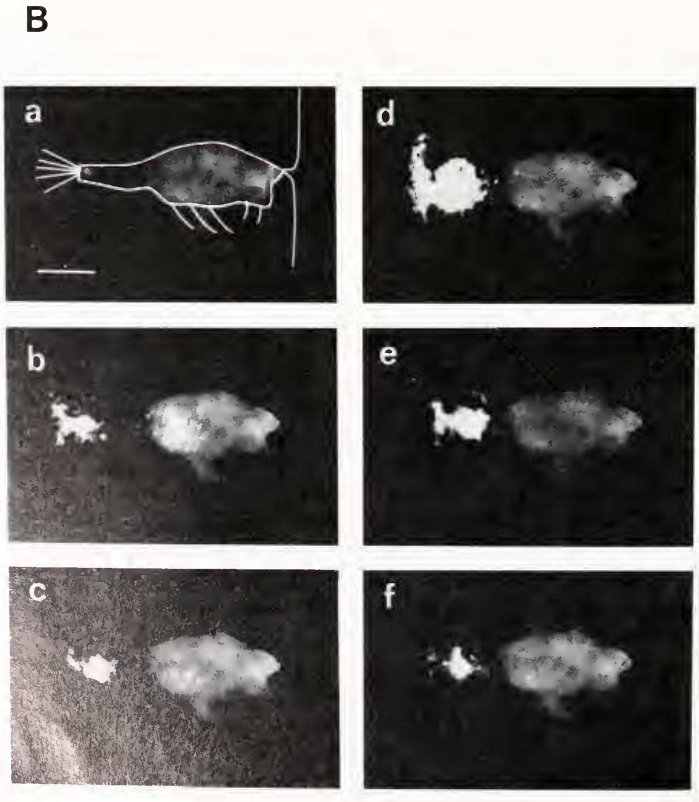
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Temporal characteristics and quantum emission

Flashes from approximately 350 specimens of *Pleuromamma xiphias* were analyzed for kinetics and quantum emission. No spontaneous flashes were observed from specimens in the apparatus prior to testing, although luminescence often was elicited by handling during preparation. Even though there was much variability in the kinetics and quantum emission of luminescent responses, it was possible to identify general trends in responsiveness.

The waveform of a flash response to a single electrical pulse (Fig. 2A) was resolved into two components. The first, characterized by fast rise and decay times, was followed, after a slight decay in intensity, by a second element consisting of a slower rise and decay (see Fig. 5B for expanded waveform). The maximum intensity of the second peak was equal to or greater than the first peak. The majority (25 out of 46) of flashes stimulated by single electrical pulses contained both components. The remaining flashes were composed of either the fast or slow component only (7 and 6 flashes, resp.), or had a waveform that was not possible to resolve (8 flashes). Quantum emissions of flashes with only one component (Fig. 2C) were from one to two orders of magnitude lower than those of two-component flashes.

Preliminary observations indicated that mechanical stimulation of light emission also elicited flashes with fast and slow components, although the kinetics and quantum emission of mechanically stimulated flashes were not investigated.

Image intensification of single specimens during the production of two-component flashes revealed the spatial pattern of emission (Fig. 3). The fast component was typically produced by a brief emission from a luminescent gland located laterally on thoracic segment 3 or 4, and simultaneous light production from another light organ located on the caudal rami (Fig. 3b). The luminescent material was not violently expelled from the body during this fast component. The decrease in light intensity following the fast component resulted from a cessation of thoracic light organ production along with a slightly diminished emission from the abdominal light organ (Fig. 3c). The slow flash component was due to increased production of luminescent material by the abdominal light organ and subsequent expulsion from the body (Fig. 3d). No other light organs were observed to be active at this time. The decay of the slow component was due to a gradual decrease in the amount of luminescent material produced by the abdominal light organ (Fig. 3e, f).

Flashes in response to a single 10 V stimulus (Table I) had a mean stimulus-response latency of 18 ms. The initial peak of the fast component occurred within 100 ms while the slower second component reached maximal intensity approximately 600 ms after the stimulus presentation. Total flash duration was 2.6 s, and the average quantum emission of a single flash was 1.4×10^{10} photons flash^{-1} (maximum of 7.1×10^{10} photons).

Decay of light emission was measured for each component of two-component flashes (n = 7). Bioluminescence decreased exponentially with time according to the

FIGURE 3. Luminescent response of a single specimen of *Pleuromamma xiphias* to a single 40 V, 5 ms electrical stimulus monitored by simultaneous image intensification and photomultiplier recording. (A) Flash waveform from the MCA showing fast and slow components. Intensity is shown as a function of time. Time bar = 2 s. (B) Simultaneous images of animal (lateral view) obtained from single frames of the video record. Scale bar = 1 mm. Letters (a) through (f) in both portions of the figure correspond to identical time periods during the flash: (a) prior to stimulation; (b) maximum emission during fast component; (c) decay in intensity of fast component; (d) maximum emission during slow component; (e) and (f) decay of slow component.

TABLE I

Response kinetics and quantum emission of flashes of Pleuromamma xiphias *stimulated with single electrical pulses (10 V, 5 ms duration)*

Latency (ms)	Total rise time (ms)		50% decay time (s)	Total flash duration (s)	Quantum emission (photons flash^{-1})
	Fast component	Slow component			
18.4*	87.1	603.0**	0.9	2.6	1.4×10^{10}
± 4.2	± 15.8	± 254.7	± 0.3	± 0.7	$\pm 0.6 \times 10^{10}$
(0.5–59)	(27–202)	(552–838)	(0.02–2.8)	(0.1–10)	$(6.1 \times 10^{7}$–$7.1 \times 10^{10})$

* Values represent the mean values ± standard errors of the mean, with ranges in parenthesis, for 14 specimens.

** Precise measurements of second component kinetics were possible in only three specimens.

standard equation for exponential decay, $Y = A * e^{(B*t)}$, where A is the y-intercept and B is the decay rate constant. Decay rates were calculated from the linear regressions of the natural logarithm of intensity with time (Fig. 2B, 2D; R = 0.99 for all regressions). The mean (±S.E.) rate of decay of the fast component of -14.6 ± 4.4 was an order of magnitude greater than the rate of decay of the second component (-1.25 ± 0.5).

A separate experiment demonstrated that flash quantum emission increased as a function of stimulus strength when animals were tested with single stimuli of 5 ms duration from 2–10 V in magnitude (Fig. 4). Minimum responses were at 2 and 4 V, where the average emission was approximately 3.8×10^9 photons flash^{-1}. Threshold

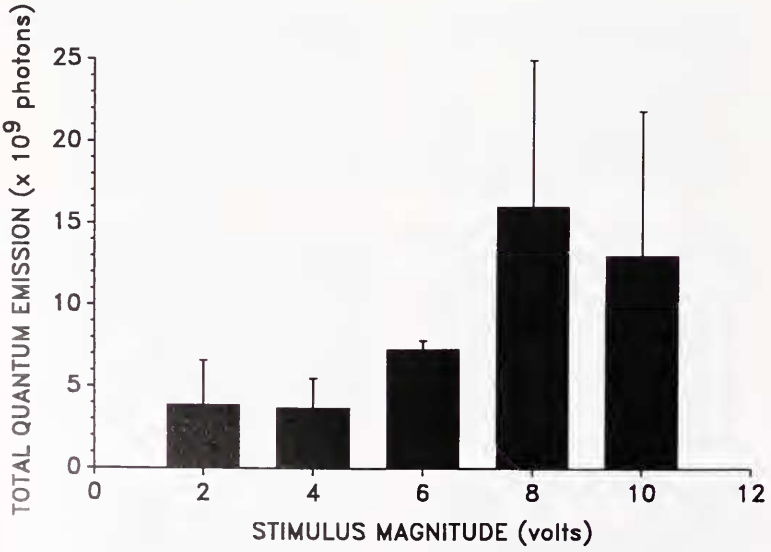

FIGURE 4. Quantum emission of *Pleuromamma xiphias* flashes as a function of the voltage of single 5 ms duration electrical pulses. For each stimulus condition, 6–7 specimens were tested. Mean values (± standard errors) are: 2 V, $3.9 \pm 2.7 \times 10^9$ photons; 4 V, $3.7 \pm 1.8 \times 10^9$ photons; 6 V, $7.3 \pm 0.5 \times 10^9$ photons; 8 V, $1.6 \pm 0.9 \times 10^{10}$ photons; 10 V, $1.3 \pm 0.9 \times 10^{10}$ photons. Due to the large standard errors, mean values were not significantly different from one another (*t*-test, $P > 0.05$), although maximum flash quantum emission at each stimulus voltage level increased.

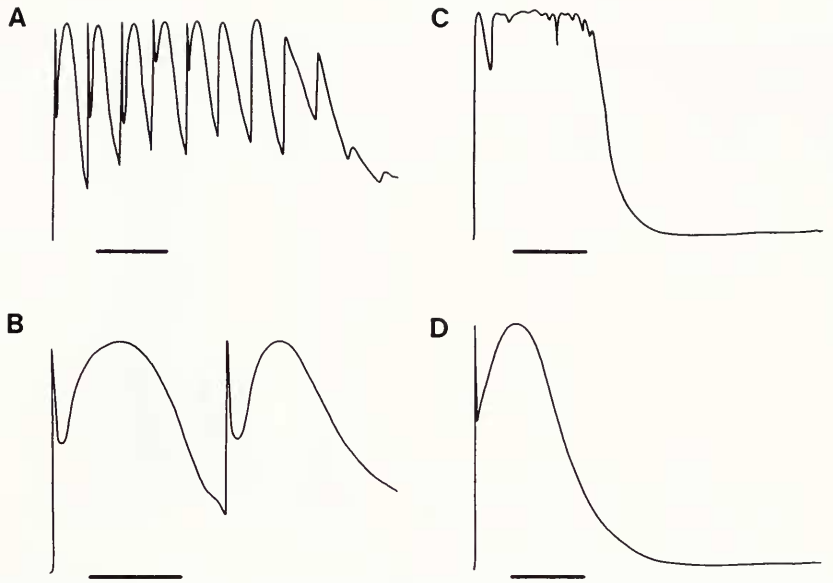

FIGURE 5. Luminescent responses of *Pleuromamma xiphias* as a function of repetitive stimulation with 10 V, 5 ms duration electrical pulses. Intensity of output (same relative scale) is displayed as a function of time. Time bars = 2 s except in (B). The maximum intensity of the responses was near the threshold of non-linearity of the detection system and may underestimate true intensity levels. (A) Response episode of one specimen to 0.5 Hz stimulation. (B) Detail of individual flashes comprising response episode for 0.5 Hz stimulation; fast and slow components of each flash are evident. Time bar = 1 s. (C) Response to 2 Hz stimulation; temporal summation produces a single prolonged response episode. (D) Response to 10 Hz stimulation. The response is similar in waveform to that for single pulse stimulation but of longer duration.

was apparently near 2 V, since at that level only 50% of the specimens tested produced luminescence, while at higher voltages all stimuli elicited a response. Stimulus voltage had no effect (Student's t-test, $P > 0.05$) on response latency (mean and standard error = 36 ± 7 ms), initial rise time (123 ± 21 ms), 50% decay time (856 ± 201 ms), or flash duration (2.8 ± 0.5 s). These values are not significantly different from those of the previous experiment with a constant 10 V stimulus (Student's t-test, $P > 0.05$).

Stimulation with repetitive pulses had the most pronounced effect on response waveform and quantum emission (Fig. 5). At the slowest stimulus rate of 0.5 Hz (Fig. 5A), the response episode consisted of individual flashes separated by partial decay of light intensity. There was a 1:1 stimulus-response correlation and no temporal summation or facilitation of single flashes. In many instances luminescence was not exhausted during the 20 s data collection period, which was equivalent to 11 stimuli; therefore the measured quantum emission underestimated the total stimulable emission. The waveform and kinetics of single flashes comprising the response episode were similar to those for single pulse stimulation; in fact it was usually possible to resolve the fast and slow flash components (Fig. 5B).

When stimulated at 1 and 2 Hz, components of individual flash responses to each stimulus pulse were still evident, but were temporally summated to form a single prolonged emission with an average duration of approximately 8 s (Fig. 5C). At 1 Hz stimulation, the average total duration of the response episode was approximately 14 s and the mean total emission was 1.1×10^{11} photons episode^{-1} (Table II). The largest response episode measured had a total emission of 2.9×10^{11} photons episode^{-1}.

Higher rates of stimulation (10 and 20 Hz) actually resulted in decreased emis-

TABLE II

Response kinetics and quantum emission of response episodes of Pleuromamma xiphias *stimulated with repetitive electrical stimuli (10 V, 5 ms duration) at frequencies ranging from 0.5 to 20 Hz*

Frequency (Hz)	Latency† (ms)	Response duration (s)	Total quantum emission (photons episode^{-1})	Maximum response (photons episode^{-1})	*n*
0.5	36.0* ± 18.2	>17.1** (2.2)†† ± 2.3 (±0.4)	5.2×10^{10}** ± 1.8×10^{10}	1.6×10^{11}**	9
1	12.2 ± 3.0	>13.8** ± 2.5	11.0×10^{10}** ± 4.9×10^{10}	2.9×10^{11}**	6
2	17.1 ± 5.4	8.1 ± 1.4	7.2×10^{10} ± 1.4×10^{10}	2.5×10^{11}	11
5	21.7 ± 6.6	8.8 ± 1.8	8.0×10^{10} ± 2.1×10^{10}	2.0×10^{11}	12
10	18.8 ± 4.3	7.5 ± 1.3	5.2×10^{10} ± 1.1×10^{10}	1.0×10^{11}	10
20	33.2 ± 8.4	5.4 ± 1.3	4.8×10^{10} ± 1.3×10^{10}	1.0×10^{11}	10

* Values represent mean ± standard error of the mean.

** The reported value underestimates the actual value since some responses persisted beyond the 20 s data collection period.

† Stimulus-response latencies for different stimulus frequencies were not significantly different from one another (*t*-test, $P > 0.05$).

†† Measurements from single flashes comprising the entire response episode.

sion; average duration of the response episode was less than 6 s with a quantum emission averaging 5×10^{10} photons episode^{-1} (Table II). Summation was so complete that individual flashes comprising the response episode were not recognizable (Fig. 5D). In fact, the resultant waveform was similar to the flash response for single pulse stimulation (Fig. 2A), although of longer duration and with greater quantum emission.

Since stimulus-response latencies were unaffected by stimulus magnitude and frequency, the values for all experiments were pooled (Fig. 6). The most common latency values occurred between 5 and 20 ms with a median latency of 16 ms (n = 152). This is similar to latencies of 7 to 9 ms reported for the Metridiidae (David and Conover, 1961; Clarke *et al.*, 1962). Minimum flash latencies of 4 to 18 ms have been reported for other species of the Metridiidae (David and Conover, 1961; Clarke *et al.*, 1962; Barnes and Case, 1972); in the present study 6% of all specimens tested exhibited latencies less than 5 ms. The briefest flash latency measured was 2 ms, probably reflecting a direct electrical effect on the light organ (Baguet, 1975; Baguet *et al.*, 1980) rather than one mediated through sensory or central pathways, since synaptic delay alone accounts for approximately 1 ms (Katz and Miledi, 1965) in chemically transmitting crustacean neuromuscular synapses.

Observations suggested that subsequent flashes from a specimen exhibited different flash kinetics and quantum emission from those of first flashes. This was tested by subjecting 5 specimens to a second 10 V, 5 ms duration pulse approximately 4 minutes following an original stimulus pulse of the same magnitude (Table III). While statistically not significantly different (*t*-test, $P > 0.05$), all specimens exhibited a second flash response that occurred after a longer latency, had a longer rise time, and

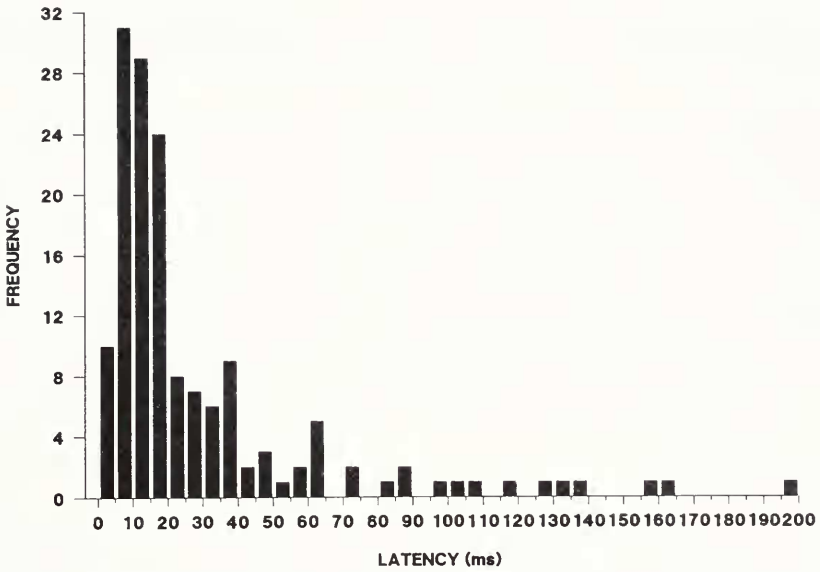

FIGURE 6. Frequency histogram of the distribution of stimulus-response latencies for 152 specimens of *Pleuromamma xiphias*. The statistical mode of the distribution was 16 ms.

was of shorter duration than the first flash. In addition there was an average 35% reduction in quantum emission for the second flash.

DISCUSSION

The most striking feature of the bioluminescence of freshly collected specimens of the copepod, *Pleuromamma xiphias,* was the variability in spectral and temporal characteristics and quantum emission of the flash response. Such variability has been

TABLE III

Comparison of response kinetics of flashes of Pleuromamma xiphias *between initial single electrical pulse (10 V, 5 ms duration) and an identical stimulus delivered 4 min later*

Latency (ms)	Total rise time (ms)	50% decay time (s)	Total flash duration (s)	Total quantum emission (photons flash^{-1})
A. First stimulus				
9.0*	103.1	1.4	3.0	1.6×10^{10}
± 2.4	± 24.2	± 0.6	± 0.6	$\pm 0.7 \times 10^{10}$
B. Second stimulus**				
21.8	212.5	0.3	2.0	0.6×10^{10}
± 5.1	± 75.6	± 0.2	± 0.6	$\pm 0.4 \times 10^{10}$

* Values represent mean ± standard error of the mean for 5 specimens.

** While mean values for flash kinetics and total quantum emission were not significantly different from those of the first flash response (*t*-test, $P > 0.05$), for each specimen the second response always had a longer latency, slower rise time, faster decay time, shorter flash duration, and diminished flash quantum emission (Sign test; Zar, 1974).

noted but not investigated in previous studies of copepod flashing (David and Conover, 1961; Clarke *et al.*, 1962; Barnes and Case, 1972; Lapota and Losee, 1984).

Emission spectra

The spectral distributions of *Pleuromamma xiphias* bioluminescence were either bimodal or unimodal. Herring (1983) measured bimodal spectra in *P. borealis,* but the luminescence for other copepod genera which have been studied exhibits unimodal distributions (David and Conover, 1961; Herring, 1983; Widder *et al.,* 1983). *P. xiphias* contains 11 luminescent glands (Giesbrecht, 1895; Clarke *et al.,* 1962) of unknown variability in spectral emissions. Since variability in the recruitment of luminescent glands is well known for copepods (David and Conover, 1961; Barnes and Case, 1972), there may be a spatial origin to the variability in the emission spectra. Observation of the sites of light emission during spectral measurements is necessary to determine whether luminescence from different body regions has different spectral properties.

Flash kinetics

Luminescence by calanoid copepods is believed to occur by the expulsion of the contents of paired sacs comprising the luminescent gland through a pore and subsequent mixing outside the body (David and Conover, 1961; Clarke *et al.,* 1962; Barnes and Case, 1972; Herring, 1985). The activation/expulsion process presumably involves at least three steps: (1) activation within the photocytes, (2) transport through internal channels, culminating in (3) expulsion through pores to outside the body.

In the present study the flash of *P. xiphias* was resolved into two components. The fast component of the flash appears to involve steps (1) and (2) above, with little or no expulsion from the body. Two glands are responsible for light emission: the caudal organ located on the posterior tip of the abdomen, and one or more of several thoracic light organs. The slow flash component consists of all three steps, with considerable spewing of luminescent material from the posterior light organ into the external environment. Although both components exhibit first order exponential decay, the different decay rates also suggest that the two components are separate events involving different emission mechanisms.

The wide range of values measured for the rise time of *P. xiphias* flashes (mean 170 ms, range 15 to 535 ms) reflect the variability known for copepods. Previous measurements for the Metridiidae range from 30 to 900 ms (Clarke *et al.,* 1962; Barnes and Case 1972; Lapota and Losee, 1984). The variability of these measurements may result from the presence of a rapid, neurally triggered response coupled with a mechanism with an inherently slower temporal element, such as glandular mixing and expulsion.

Flash durations for *Pleuromamma xiphias* were stimulus-dependent and extended over 2 orders of magnitude, ranging from 100 ms to greater than 20 s. Previous measurements of flash durations for *Pleuromamma* species range from 200 ms to over 6 s (Clarke *et al.,* 1962; Yevstigneyev, 1985), and 100 ms to 11 s for other copepods (Barnes and Case, 1972; Lapota and Losee, 1984). In this study the maximum period over which luminescence could be expressed was not investigated; however, the copepod *Gaussia* is able to respond to single electrical pulses delivered at 0.1 Hz for more than 3 min (Barnes and Case, 1972).

Quantum emission

Considering the abundance of luminescent plankton, there are few measurements available of the quantum emission of individual flashes, in part due to the difficulty

TABLE IV

Total quantum emission of single flashes from planktonic organisms

Organism	Mode of stimulation	Mean total quantum emission (photons flash^{-1})	Reference
Protozoa			
Colonial radiolaria	Mechanical	1×10^9	Latz *et al.*, 1987
Dinophyta			
Noctiluca miliaris	Electrical	2×10^9	Eckert, 1967
Ceratium horridum	Vacuum*	4×10^7	Lapota and Losee, 1984
Ceratium breve	Vacuum	1×10^8	Lapota and Losee, 1984
Pyrocystis noctiluca	Mechanical	7×10^9	Latz and Case, unpub.
Pyrocystis fusiformis	Mechanical	2×10^{10} first flash 0.1×10^{10} subsequent flash	Latz and Case, unpub.
Gonyaulax polyedra	Spontaneous	1×10^7	Latz and Case, unpub.
Crustacea			
Euphausiacea			
Euphausia eximia calyptopis I	Vacuum	1×10^{10}	Lapota and Losee, 1984
Nyctiphanes simplex furcilia I	Vacuum	6×10^{10}	Lapota and Losee, 1984
N. simplex furcilia III	Vacuum	1×10^{11}	Lapota and Losee, 1984
Copepoda			
Pleuromamma xiphias	Single electrical Repetitive elect.	1×10^{10} 9×10^{10}	Present study
Corycaeus latus	Vacuum	1×10^8	Lapota and Losee, 1984
Centropages furcatus	Vacuum	8×10^7	Lapota and Losee, 1984
Corycaeus speciousus	Vacuum	5×10^7	Lapota and Losee, 1984
Paracalanus indicus	Vacuum	3×10^7	Lapota and Losee, 1984
Ostracoda			
Conchoecia secernenda	Electrical	3×10^{10}	Latz, Frank, and Case, unpubl.

* Flashes were induced by removing water from chamber, stranding organisms on filter paper.

in making these measurements. Electrical stimulation allows for precise control of stimulus parameters such as pulse strength, duration, and frequency, and the results of the present study have demonstrated that changes in these parameters greatly influence the resulting quantum emission of the luminescent responses. Quiescent specimens of *P. xiphias* were extremely sensitive to handling; it was not uncommon for some bioluminescence to be triggered during handling. Therefore, the present measurements of flash output are conservative estimates of luminescence capacity.

Light emission by planktonic organisms for which data are available (Table IV) ranges from 1×10^7 photons flash^{-1} for the dinoflagellate *Gonyaulax* (Latz and Case, unpub.) to approximately 1×10^{11} photons flash^{-1} for larval euphausiids (Lapota and Losee, 1984). The quantum emission of luminescence by *P. xiphias* is situated at the upper portion of this range, and is 1.5–2.5 orders of magnitude greater than that reported for smaller copepods by Lapota and Losee (1984), although these differences may also reflect differing excitational and recording methods as well as species differences.

The present data on the total quantum emission of *Pleuromamma xiphias* luminescence reflect the variability in responses previously observed in the Metridiidae (David and Conover, 1961; Clarke *et al.*, 1962; Barnes and Case, 1972). The maximum total quantum emission measured following electrical stimulation was equivalent to the bioluminescence potential, or total luminescent capacity of *P. xiphias*, which, based on measurements of total mechanically stimulated bioluminescence, is estimated to be approximately 1×10^{11} photons (Latz and Case, unpub.). Therefore

a single flash represented approximately 4–15% of total possible light emission. Thus, *P. xiphias*, as well as other copepods, is capable of emitting numerous flashes before response failure occurs (Clarke *et al.*, 1962; Barnes and Case, 1972; present study). In the present study, electrical stimulation at 2 Hz effectively evoked the total luminescent capacity, while other stimulus frequencies generated flash episodes which utilized 50% or more of total luminescent capacity.

Implications of flash variability

Previous studies of copepod luminescence have noted the "irregular" shapes of the flash responses (Clarke *et al.*, 1962; Barnes and Case, 1972). Generally, response episodes to repetitive stimulation exhibited more complex and variable waveforms than flashes induced by single stimuli. This trend was also true for *P. xiphias*. For this organism, stimulus frequency was a predictable source of variability of the response waveform. However, the general pattern was the higher the stimulus frequency, the simpler the flash waveform, due in part to different degrees of temporal summation of individual flashes comprising the response episode. There was no standard response waveform for all stimulus protocols.

Such a large capacity for luminescence, plus the variability in flash waveform and quantum emission, suggest that copepods are not limited to a single type of flash but instead may exhibit a versatility of luminescent behaviors. Behavioral versatility in zooplankton-secreted luminescence is in fact known for coastal ostracods, for which three types of behaviorally significant luminescent displays have been described (Morin and Bermingham, 1980; Morin, 1986). These displays involve not only control of the interval between flashes but also of flash duration, the latter through mechanisms that may involve differences in the chemical composition of the luminescent secretion. In some species individual ostrocods produce dozens of flashes during the signaling sequence. Evidence for behavioral versatility of luminescent displays also exists for squid (Young *et al.*, 1982), fish (McFall-Ngai and Dunlap, 1983), and for counter-illuminating organisms (reviewed by Young, 1983).

There is evidence for multiple modes of copepod luminescence. *Metridia* generates two types of flashes during feeding experiments with euphausiids as predators (David and Conover, 1961). Single bright flashes may be associated with an escape response, while multiple flashes over a 30-s interval may be associated with successful predation.

In terms of the present measurements of the physical characteristics of the flash of *Pleuromamma xiphias*, variability in the emission spectra, flash kinetics and flash quantum emission most likely result from a combination of several factors: spatial origin of emission from the body, temporal summation and complex neural processes (as determined by stimulus strength and frequency), previous excitation history, collection and handling artifacts, and general physiological state of the organism. Until additional experiments can correlate flash patterns with specific behaviors, the variability in bioluminescence of *P. xiphias* only suggests the physiological mechanisms responsible for possible differences in luminescent displays.

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